THE EFFECT OF VITAMINS OF THE B COMPLEX ON THE RESISTANCE OF THE ORGANISM TO ANOXIA

BY

HAROLD FRANK HAILMAN

B.S., University of Illinois, 1938
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AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHYSIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1943

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Previous studies have shown that certain vitamins of the B complex play a role in cellular oxidation and that, in addition, one of them, riboflavin, is able to prevent the negative inotropic effect of anoxia on cardiac muscle. Therefore it seemed worthy to study the effect of these vitamins on the resistance of the organism to lack of oxygen, particularly the central nervous system and the heart, since these are the parts of the body from which arise most of the symptoms and practically all of the dangers of anoxia.

Because these experiments demonstrated that riboflavin prevented the effects of anoxia on reflex nervous activity, it was decided to determine whether or not riboflavin has an anti-anoxic effect on some less specialized cell. The tissue chosen for this study was the smooth muscle of the frog's esophagus.

Since anoxia is also known to cause hypertrophy of the adrenal cortex, the ability of the vitamins of the B complex to prevent this increase in adrenal weight was also studied. Further studies were made on the mechanism of adrenal cortical hypertrophy in which the effect of vagotomy on this phenomenon was studied in rats. These latter studies were carried out because injections of insulin have been shown to cause adrenal hypertrophy and because the vagus nerves control insulin secretion in anoxia.

In addition, a small group of experiments was carried out in man in which the ability of the vitamins of the B group to offset the effects of anoxia on the critical fusion frequency, electroencephalogram, and pulse rate was studied.

Methods

Linguo-maxillary Reflex

This reflex was selected for the purpose of studying the effects of the vitamins of the B complex on the resistance of the central nervous system to anoxia. This reflex consists of a contraction of the anterior belly of the digastric muscle upon stimulation of the tongue.

Acute experiments were performed upon 18 dogs weighing approximately 10 kg. The animals were anesthetized with sodium amytal, 60 mg. per kg., or with a combination of sodium amytal, 40 mg. per kg., and chloralose, 20 mg. per kg. Blood pressure was recorded from the femoral artery by means of a mercury manometer. The trachea was cannulated and connected to an electrical respiratory pump with adjustable stroke volume. Gas mixtures were inhaled from Douglas bags, filled by a calli-

brated flow meter. Intravenous injections were made into the cannulated femoral vein.

The preparation and method of recording the response of the linguomaxillary reflex was as follows: The tendon of the anterior belly of the digastric muscle was separated from its insertion upon the inferior border of the mandible, and the muscle freed for about one-half its length from the adjoining muscles and fascia. The freed end was attached by a thread to the rubber dam of a tambour, which in turn was attached to a recording tambour by rubber tubing. A contraction of the muscle caused a decrease in the pressure in the system and resulted in a down stroke of the recording lever. The preparation was shielded by gauze sponges kept moist with Ringer's solution and warmed by an electric light bulb. The animal's head was immobilized in a head holder to prevent the recording of extraneous movements. Stimulation was accomplished by electrodes fastened to the tongue, and a current was supplied by a condenser discharging at a rate of one per second.

Denervation of the carotid sinuses, when necessary, was done by stripping the adventitia from the vessels one centimeter above and below the carotid bifurcation and applying to the area 5 per cent phenol, followed by 95 per cent alcohol. The vagi were cut routinely with this procedure to eliminate the pressure and chemoreceptor zones of the aortic arch and body.

Frog Heart

The preparation used to study the effect of the vitamins of the B group on cardiac muscle under conditions of anoxia was the Straub preparation of the frog heart. In this preparation, after pithing the frog, the aorta was opened near the heart and a fine glass cannula was inserted into the aorta and then gently manoeuvred down into the ventricle of the heart where it was tied in place. The great veins entering the heart were tied off and the heart was removed from the body of the frog and placed in a small glass chamber where the air was kept moist by means of wet filter paper. One cubic centimeter of a phosphate-buffered Ringer's solution (pH 7.2) containing 0.5 per cent glucose was then placed in the cannula and allowed to flow to and fro into the chambers of the heart as a result of their spontaneous rhythmical contractions. One end of a fine capillary tube was placed below the surface of the solution and the other end was connected to a "Y" tube so that the solution could be perfused with either air or nitrogen. The tip of the ventricle was tied by means of a fine waxed silk thread to a frog heart lever, in order to record the cardiac contractions on a slowly rotating smoked drum.

The experiments were carried out as follows: After a control record

of cardiac contractions was made in the presence of air, the drum was stopped and the air was replaced by nitrogen. After a significant depression of the height of the cardiac contractions appeared, which usually occurred in from three to five minutes, the drum was again started and the vitamin to be tested was immediately added to the perfusion fluid. After five minutes, the nitrogen was replaced by air and the experiment terminated two to three minutes later.

Smooth Muscle

The frog was doubly pithed and a 5 mm. section of the esophagus was removed. The wall was cut through at one point and the strip suspended between two hooks and fastened to a lever so that the spontaneous contractions of the circular muscle could be recorded on a moving kymograph. The strip was kept in a bath of a phosphate-buffered Ringer's solution (pH 7.2) containing 0.5 per cent glucose, which could be perfused with either air or nitrogen. After a control experiment was performed in which the effect of nitrogen in the absence of riboflavin on the spontaneous muscular contractions was determined, air was allowed to replace the nitrogen and the suppressed contractions returned to normal. Riboflavin in a concentration of 1 to 100,000 was then added to the Ringer's solution and the nitrogen perfusion was repeated 15 minutes later. This interval was allowed so that the riboflavin could be converted into the functioning respiratory enzyme by the time the nitrogen effect had become maximal, as determined by the control experiment. The riboflavin was then removed and the experiment was repeated in riboflavin-free Ringer's solution for a second control.

Adrenal Cortex

Forty-five male rats weighing 200 to 250 grams each were divided into five groups of nine each. The first group was used as a control group; the second group was not allowed to eat or drink for 48 hours; the third group was subjected to a barometric pressure of 294 mm. Hg for 48 hours, during which time the animals did not eat or drink; the fourth group was injected daily with riboflavin, 1 mg. per 250 grams, for six days and then subjected to the same reduced pressure as group 3; and the fifth group was first vagotomized and also subjected to the same reduced pressure. The animals of each group were then sacrificed and the adrenal glands removed and weighed.

An additional group of four animals received all the vitamins belonging to the B complex for six days and were then subjected to a reduced barometric pressure of 294 mm. Hg for 48 hours, as were the other

groups. Each rat received one ampule of the B complex[1] per day for a period of six days; it was administered in the form of a suspension by means of a stomach tube. Each ampule contained the following vitamins: thiamin, 1 milligram; riboflavin, 333 micrograms; nicotinic acid, 5 milligrams; pyridoxine, 200 micrograms; pantothenic acid, 250 micrograms; and liver concentrate.

The vagotomies were performed under ether anesthesia. An incision was made on the left side of the abdomen just below the rib margin, and the peritoneal cavity was entered. The intra-abdominal portion of the esophagus was isolated and the right and left vagi cut. The esophagus was then painted with 5 per cent phenol and washed with alcohol. The viscera were returned to their proper position and the abdomen closed in layers.

Experiments on Man

(Critical Fusion Frequency, Electroencephalogram, and Pulse Rate)

In this group of experiments the effects of anoxia on the critical fusion frequency, electroencephalogram, and pulse rate were studied before and after the administration of relatively large doses of the vitamins of the B group.

For the determination of the critical fusion frequency (C.F.F.) a neon bulb whose flickering frequency could be controlled by an audiometer served as a source of flickering light. The area of the test field exposed to the eye equaled one degree, and when the flicker frequency was not being determined the test field was covered by a screen. The subject observed the test field through an artificial pupil so as to avoid any variability resulting from changes in pupillary diameter. Only one eye was used, the other being covered. At the appropriate signal the subject raised the screen and observed the test field while the experimenter gradually lowered the flicker frequency. When the first trace of flicker was observed the subject would indicate this by tapping the table and promptly lowering the screen. After a short practice period it was possible for the subject to give very consistent results, individual readings varying by not more than one flash per second. The readings were always taken in groups of two or three and the results averaged.

During the intervals when the C.F.F. was not being measured, records were made of the electroencephalogram (E.E.G.) and the pulse rate. By means of bipolar leads the occipital alpha rhythm was recorded with the eyes closed. In addition, the effect of object fixation on the E.E.G. under conditions of anoxia was likewise studied before and after the

[1] Kindly supplied by Abbott Laboratories.

administration of the vitamins of the B complex. The pulse rate was recorded electrically with leads from the left forearm and leg.

The anoxia was produced by having the subjects breathe hypoxic gas mixtures (7 to 8.4% oxygen) from Douglas bags through an oro-nasal oxygen mask. A system of two valves permitted the subject to inhale from the Douglas bags and exhale to the outside room air. The period of low oxygen inhalation was maintained until significant changes occurred in the C.F.F. and E.E.G., which usually required 10 to 15 minutes.

After the control effects were obtained, the following amounts of vitamins were given to the subjects daily for a period of three weeks: thiamin, 6 milligrams; riboflavin, 12.6 milligrams; nicotinamide, 40 milligrams; pyridoxine, .8 milligrams; pantothenic acid, 2 milligrams; and also other factors from 12 grains of yeast.

After the three-week period the subjects were again tested and the effects of anoxia on the C.F.F. and E.E.G. were recorded.

Results

Linguo-maxillary Reflex

Control Experiments. — In a group of five control experiments, periods of anoxia were repeated at 15-minute intervals with no vitamins being given, in order to determine the constancy of the effect of anoxia on the reflex. The concentrations of oxygen used varied between 4.5 per cent and 9.1 per cent, and the duration of the inhalation of the anoxic gas mixture was determined by the response of the linguo-maxillary reflex. The degree and duration of the anoxic period was that necessary to produce a definite depression of the reflex. All control experiments showed that the depression remained constant or became more severe, and in no case did the anoxic effect become less.

Experiments with Riboflavin. — A series of 12 experiments on seven dogs was performed in which the effect of riboflavin, 1 mg. per kg., on the anoxic depression of the linguo-maxillary reflex was studied. These experiments all showed that riboflavin prevented the depressing effect of anoxia on the reflex.

After it was shown that at least two periods of anoxia, repeated at 15-minute intervals, caused a constant and definite depression of the linguo-maxillary reflex, riboflavin, 1 mg. per kg., was given intravenously. The anoxia was then repeated at the same interval for one or more hours. In all cases the anoxic effect on the reflex became less. However, it was interesting to note that the maximal anti-anoxic effect was not obtained until approximately 45 minutes after the administration of riboflavin.

TABLE I

THE ANTI-ANOXIC EFFECT OF RIBOFLAVIN ON THE LINGUO-MAXILLARY REFLEX

Dose = 1 mg. Riboflavin per kg., Given Intravenously

Experiment	Normal or Denervated	Degree of Anoxia (%)	Duration of Anoxia (min.)	Height of Reflex Contractions During Anoxia in Per Cent of Control in Air				
				Before Riboflavin (%)	15 Min. After Riboflavin (%)	30 Min. After Riboflavin (%)	45 Min. After Riboflavin (%)	60 Min. After Riboflavin (%)
1	N	6.2	2	0	10	40	100	..
2	N	6.2	5	0	0	50	..	33
3	N	6.2	2	0	35	50	..	..
4	N	5.7	2	0 (88 sec.)*	0 (63 sec.)	0 (13 sec.)	0 (13 sec.)	0 (189+ sec.)
5	N	5.7	2	0 (160+ sec.)*	40	0 (32 sec.)	60	100
6	D	6.2	2	50	60	100	100	100
7	D	4.5	3½	40	40	50	100	..
8	D	4.5	3½	40	60	..	..	100
9	D	6.2	4	30	83	..	62	40
10	D	6.2	4	0	0	20	50	36
11	D	6.2	4	0	..	0	30	50
12	D	4.5	2	0 (66 sec.)*	0 (53 sec.)	0 (50 sec.)	0 (28 sec.)	0 (7 sec.)

* In experiments 4, 5, and 12 the riboflavin did not prevent the abolishment of the reflex during anoxia, but it did shorten the duration of the complete suppression as shown by the time in seconds.

Similar results were obtained in dogs whose carotid sinuses had been denervated and vagi cut. Table I summarizes this group of experiments.

Experiments with Nicotinamide and Thiamin. — The same type of experiment as described for riboflavin was performed, using thiamin or nicotinamide, 5 mg. per kg. and 10 mg. per kg. respectively. Neither of these vitamins had any effect in preventing the depression of reflex activity by anoxia.

Frog Heart. — These experiments failed to show any beneficial effect of the vitamins of the B group on the reduction of the strength of cardiac contractions produced by anoxia. Riboflavin, thiamin, phosphorylated thiamin, and nicotinamide were used in concentrations varying between 1 to 10,000 and 1 to 200,000. However, the readmission of air promptly restored the height of contractions to the control level.

Smooth Muscle. — The experiments performed on an isolated strip of smooth muscle show that riboflavin is largely able to prevent the suppression of spontaneous rhythmical contractions caused by anoxia. It was shown that 15 to 25 minutes' perfusion of the smooth muscle preparation with nitrogen in the absence of riboflavin caused a marked depression of the strength of the muscular contraction. However, when the same procedure was carried out in the presence of riboflavin in a concentration of 1 to 100,000, the anoxic effect was greatly reduced. These results were uniformly obtained on six preparations.

Adrenal Cortex. — The results given in Table II show primarily that riboflavin, the entire B complex, and vagotomy had no effect on the hypertrophy of the adrenal due to anoxia. The group from which food and water were withheld for 48 hours showed no change from the control

TABLE II

Effect of 48 Hours Exposure to a Barometric Pressure of 294 mm. Hg on the Adrenal Weight of Rats

(Weights Given in Milligrams)

Rat	Control Group	No Food or Water for 48 Hrs.	294 mm. Hg 48 Hrs.	294 mm. Hg 48 Hrs. Riboflavin	294 mm. Hg 48 Hrs. B Complex	294 mm. Hg 48 Hrs. Vagotomized
1	20	26	33	30	46	36
2	19	30	39	35	30	38
3	20	29	33	34	37	31
4	26	25	32	32	46	31
5	28	25	31	31	..	30
6	24	21	39	39	..	46
7	31	24	38	33	..	31
8	25	23	33	38	..	39
9	26	25	31	28	..	39
Average	24.3	25.3	34.3	33.3	39.7	35.66

group in adrenal weight; thus, the increase in weight which occurred in the groups subjected to reduced barometric pressure was due to this latter factor and not to the fact that they did not eat or drink. The average weight of both adrenals for this group was 25.3 mg. with variations between 21 mg. and 30 mg.

The third group, which was subjected to a barometric pressure of 294 mm. Hg for 48 hours, showed an average adrenal weight of 34.3 mg. with variations between 31 mg. and 39 mg., or a 36 per cent increase. The group which received riboflavin for six days prior to the 48-hour-period of anoxia also showed an increase comparable to group three, the average weight being 33.3 mg. with variations between 28 mg. and 39 mg. The group receiving the B complex had an average adrenal weight of 39.7 mg. with variations between 30 mg. and 46 mg., and the vagotomized group had an average adrenal weight of 35.66 mg. with variations between 30 mg. and 46 mg.

Experiments on Man (C.F.F., E.E.G., and Pulse Rate). — The inhalation of 7.0 to 8.4 per cent oxygen for 10 to 15 minutes caused a fall in C.F.F. of 5 to 10 flashes per second, a diminution of the effect of object fixation on the occipital alpha rhythm of the E.E.G., the appearance of delta waves in the E.E.G., and a rise in pulse of 30 to 50 beats per minute. These changes could not be prevented by the relatively large doses of vitamins of the B complex given over a 3-week-period and in one case for 5 weeks. These findings were obtained from 4 subjects.

Conclusions

Of the three vitamins of the B group studied here, riboflavin, thiamin, and nicotinamide, only riboflavin was shown to prevent the effects of anoxia. It is believed that the anti-anoxic effect of riboflavin on the linguo-maxillary reflex is due to improved conditions for cellular oxidation and not to an improved circulation. This is borne out by the fact that the anti-anoxic effect on the reflex is not correlated with an increased blood pressure response and also by the fact that it occurred in animals whose chemoreceptor zones were removed. In addition, the ability of riboflavin to prevent the depressing effect of anoxia on the spontaneous rhythmical contractions of smooth muscle also tends to substantiate the concept that the anti-anoxic effect of riboflavin is a general cellular effect and is not restricted to a particular tissue or organ.

The failure to confirm the results of a previous investigation,[2] in which riboflavin was shown to prevent the negative inotropic effect of anoxia on cardiac muscle, might have been due to the fact that phospho-

[2] Dietrich, S., and Pendl, E.: *Klin. Wochen.*, 16:13, 1937.

rylated riboflavin may have been used but not mentioned. Several additional experiments were therefore carried out in which the phosphorylated compound of riboflavin was used. Negative results were also obtained with this substance; hence the inability to confirm Dietrich's results remains unexplained.

The inability of the vitamins of the B group and vagotomy to prevent adrenal cortical hypertrophy may be due to the fact that the conditions of the experiment were too severe, since a hypertrophy of 36 per cent occurred in only 48 hours of anoxia. It is possible that under more mild conditions of anoxia the vitamins of the B group may be effective. Regarding the mechanism of hypertrophy of the adrenal cortex, under the relatively severe conditions of this experiment, it may be that a second factor (hypophysis) was operative in addition to insulin, and hence vagotomy had no effect.

In view of the fact that riboflavin offset the effects of anoxia on the linguo-maxillary reflex in the dog, the failure of the vitamins of the B group to prevent the changes due to anoxia on the E.E.G. and C.F.F. in man is difficult to explain. One reason may possibly be the relatively larger dose of riboflavin given in the former case.

APPENDIX

Part I. Studies on the Effect of Decreased Barometric Pressure on the Electroencephalogram

Two factors are operative under conditions of decreased barometric pressure; one is anoxia, and the other is the decreased pressure per se. Experiments were performed to determine the relative role of each factor in causing changes in the electroencephalogram under conditions of decreased barometric pressure.

These experiments performed on unanesthetized rats showed that when the barometric pressure was reduced to 130 mm. Hg in the presence of 100 per cent oxygen, no changes in the electroencephalogram occurred, but when the same procedure was performed in the presence of air, there was a complete disappearance of all brain potentials. Hence the mechanical factor of reduced pressure under these conditions has no effect on the electroencephalogram.

Further studies were made on the ability of benzedrine to prevent changes in the electroencephalogram caused by a decrease in barometric pressure in the presence of air to 190 mm. Hg which was maintained for 30 minutes. It was found that the electroencephalogram of untreated rats showed a temporary suppression of all electrical activity after two to three minutes at a barometric pressure of 190 mm. Hg. Several minutes later the electrical activity reappeared, and at 10 to 15 minutes "spindles" occurred coincident with a drowsy state of the animal.

Rats treated with benzedrine (5 mg. per kg., intraperitoneally) 30 minutes before being subjected to the same reduced pressure of 190 mm. Hg also showed the initial temporary suppression of electrical activity, but following the recovery, the electroencephalogram showed the normal alpha rhythm, and in no case was the subsequent spindling[1] or drowsiness observed.

Part II. Lowest Barometric Pressure Compatible with Life in an Atmosphere of 100 Per Cent Oxygen

It has been stated that life is impossible at an altitude where the barometric pressure equals 87 mm. Hg even in an atmosphere of pure oxygen. This conclusion was based on the assumption that the sum of the partial pressures of water vapor and carbon dioxide equaled 87 mm. Hg. It has been shown, however, that both the carbon dioxide and water vapor tensions fall during hyper-ventilation; therefore, the figure of 87 mm. Hg seemed too high. The present experiments were designed to determine the lowest barometric pressure compatible with life in an atmosphere of 100 per cent oxygen.

Male rats were subjected to a gradual reduction in barometric pressure in the presence of pure oxygen and the level at which gasping occurred was noted. It was found that the average level was 63 mm. Hg with variations between 70 and 50 mm. Hg.

These results indicate that the partial pressures of carbon dioxide and water vapor fall lower than previously assumed, and therefore life is possible at a higher altitude in the presence of pure oxygen than heretofore believed.

[1] The term "spindle" is used to denote periodic outbursts of waves whose frequency is about 12 to 14 per second and whose maximal amplitude is about 150 microvolts.

VITA

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